RHYNCHOSPOROSIDE BINDING PROTEINS OF BARLEY

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1. Introduction

Rhynchosporium secalis (Oud.) Davis causes the scald disease in barley and several other grasses. The fungus establishes a subcuticular mycelium prior to invasion of the tissues [1,2]. Eventually, an increase in the permeability of the underlying host cells occurs with disruption of the plasmalemma and symptoms appear [3,4]. Cell disruption may be caused by the toxin, rhynchosporoside, one of the 1-*O*-α-cellobiosides of 1,2-propanediol [5,6]. One explanation for the selectivity of fungal phytotoxins is that the susceptible host possesses protein receptors, which, when interacting with the toxin, undergo a conformational change that modifies one or more key metabolic processes in the cell [7].

We demonstrate here the isolation and characterization of rhynchosporoside-binding proteins from crude membrane preparations of barley cultivars either sensitive or insensitive to rhynchosporoside. Furthermore, we relate these findings to the involvement of these proteins in the host–parasite interaction.

2. Materials and methods

Two near isogenic lines of Betzes barley *Hordeum* (*vulgare* L.) were used, one resistant (toxin-insensitive) and susceptible (toxin-sensitive) to scald disease. All plants tested in the bioassay system were 9 days old. The plants were reared as in [5].

[^{14}C]Rhynchosporoside with spec. radioact. 7.9 μCi/mmol was prepared by incubating *R. secalis* with [^{14}C]glucose 0.25 mg/0.25 mCi [5].

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[^{14}C]Cellobiose, 100 μCi/mmol, was obtained by incubating [^{14}C]cellulose with 0.25 mg purified cellulase in 1 ml 0.2 M acetate buffer (pH 5.4) at 36°C for 12 h. The radiolabeled cellobiose was obtained after paper chromatography of the reaction mixture in *n*-butanol–pyridine–H_2O 9:5:4, v/v/v. [^{14}C]Glucose, 102 μCi/mmol was purchased from New England Nuclear Corp.

Crude preparations of barley membranes were obtained as in [7]. The crude membranes from 100 g leaves were resuspended in 25 ml 50 mM Tris–HCl (pH 7.4, standard) containing 0.5 M trichloroacetate (pH adjusted with NaOH). The suspended membranes were incubated at 4°C for 1 h, then centrifuged at 48 000 × *g* for 30 min. The supernatant liquid was applied to a 15 × 3.6 cm affinity column prepared as in [8] except that Sepharose 4B was used as the matrix gel and cellobiose was the sugar immobilized by the vinylsulfone bridges. The affinity column was rinsed with the standard buffer solution, then the protein was eluted with 25 ml 100 mM cellobiose. The protein in the first 175 ml from the column was lyophilized and resuspended in 1.0 ml of standard buffer. Cellobiose was removed by passing the resuspended protein through a 60 × 1.4 cm column of Bio Gel P-4. Protein concentrations were measured according to [9,10].

Binding assays were performed by incubating membranes, or purified membrane proteins with 0.25 mM radiolabeled substrate in 2 ml standard buffer at 23°C for 2 h. The reaction mixture was applied to a 60 × 1.4 cm column of Bio Gel P-4 and eluted with standard buffer. The total radioactivity appearing at the void volume of the column was taken as the amount of substrate bound.

Gel electrophoresis of the binding proteins was performed on 7.5% acrylamide gels, at pH 8.3 [11]. Sodium dodecyl sulfate (SDS) gels were run at pH 7.0 [12] except that the electrophoresis buffer was diluted 4-fold and the gels were 13 cm long.

3. Results and discussion

The crude membrane preparation of resistant barley bound 2.7 nmol rhynchosporoside/mg protein, whereas the susceptible barley bound 5.5 nmol/mg protein. This result was consistent in at least 3 separate experiments. No binding activity was present in the soluble extract (supernatant liquid) of the leaves. When the crude barley membrane preparation from 100 g of either resistant or susceptible leaves was extracted by trichloracetate treatment, about 20 mg soluble protein was obtained. After affinity chromatography ~40 μg protein was recovered and this represented a 26-fold increase in the specific binding activity compared to the crude membrane preparation (table 1).

The specific binding activity of proteins from susceptible and resistant barley may mean that qualitative or quantative differences exist in the binding proteins. Gel electrophoresis of the purified binding proteins from resistant and susceptible plants indicated that they both possess a major band and a trace of another band. The relative electrophoretic mobilities of the bands from the resistant and susceptible cultivars were quite similar. Nevertheless, these proteins differed in their substrate specificities. The protein from the susceptible cultivar possessed a greater binding activity for the substrates tested (table 1).

The SDS–gel of the binding protein from the susceptible cultivar showed a major band corresponding to 82 000 daltons, another band at 63 000 daltons, and a minor band at 16 000 daltons (fig.1). The bind-

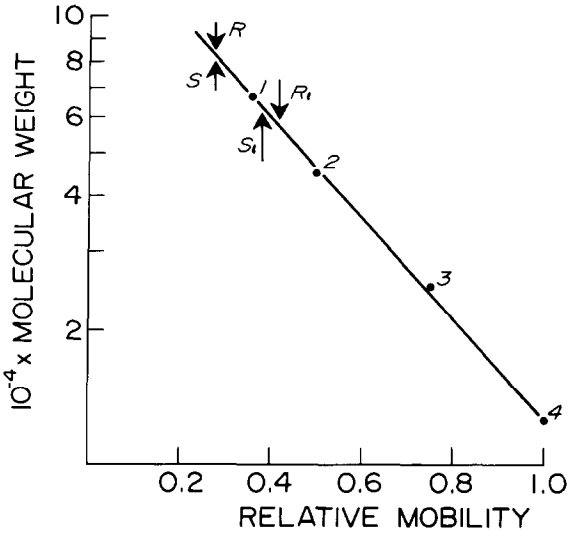

Fig.1. A plot of molecular weight as a function of protein mobility on SDS gels: R, toxin-binding protein from resistant Betzes; R, 57 000 dalton form; S, toxin-binding protein from susceptible Betzes; S_1 63 000 dalton form. The standard proteins are: 1, bovine serum albumin; 2, egg albumin; 3, chymotrypsinogen; 4, cytochrome *c*.

Table 1
Binding activities of the proteins from barley membranes

Substrate	Specific binding activity[a]	
	Resistant (nmol substrate/mg protein)	Susceptible
[^{14}C]Rhynchosporoside	68.9	151.3
[^{14}C]Glucose	21.4	34.6
[^{14}C]Cellobiose	4.8	15.9

[a] Purified binding protein (50 μg) in 2 ml standard buffer were incubated with the radioactive substrates at 0.25 mM at 23°C for 2 h. Then the reaction mixtures were applied to a Bio Gel P-4 column and the radioactivity at the void volume taken as the amount of substrate bound. Results are an average of 2 determinations

ing protein from the resistant cultivar also showed a major band at 82 000 daltons and another band corresponding to 57 000 daltons (fig.1). Thus, the toxin-binding protein in both cultivars probably exists in the native state at 82 000 daltons since this band is the most prevalent in both preparations. Nevertheless, we suggest that this toxin-binding protein in both cultivars consists of multiple subunits and that the subunit composition of the two proteins is different. In one possible subunit arrangement, the binding protein from the susceptible cultivar may contain 5 subunits, whereas the binding protein from the resistant clone may contain 6 subunits.

The differences in the physical properties of the two proteins might be used to explain their involvement in the sensitivity of these barley cultivars to the glycosidic toxin of *R. secalis* [7]. Some support for this hypothesis was obtained from in vivo toxin treatment experiments utilizing 1 ml 60 μg toxin/ml solution and the susceptible Betzes cultivar. Normally, leaves of susceptible Betzes seedlings wilt and die at the leaf tip [5]. In repeat experiments (60 μg toxin/ml), toxin-induced symptoms were apparent, 12 h after toxin application. However, susceptible Betzes seedlings preincubated in 10 mM glucose or 10 mM cellobiose for 24 h, then placed in the toxin solution (without the sugar), did not show toxin-induced symptoms until 27 h after toxin application. Galactose (10 mM) had no protective effect. The results suggest that cellobiose and glucose effectively competed for toxin-binding sites, thus temporarily precluding the development of symptoms. This would be expected since glucose and cellobiose are structurally related to the toxin. These experiments are similar to the studies [7] on the effects of α-galactosides in delaying helminthosporoside-induced symptoms in susceptible sugarcane clones.

In the in vitro binding assay, cellobiose (10 mM) completely inhibited toxin binding in both resistant and susceptible cultivars, whereas glucose (10 mM) inhibited toxin binding by 17% in both cultivars. Since the in vitro binding data are related to the effects of these sugars in vivo, we tentatively conclude that the toxin-binding protein plays some role in the sensitivity of the susceptible Betzes cultivar to rhynchosporoside. Binding site kinetics, and specificity data on various sugar linkages will be needed for additional support of this hypothesis.

Furthermore, since the binding protein from resistant plants also binds the toxin (table 1), the resistant Betzes cultivar would also be expected to show sensitivity to the toxin under some conditions. For instance, if the resistant Betzes is exposed to 500 μg toxin/ml typical toxin-induced symptoms are expressed. This result is expected since the toxin-binding protein from resistant Betzes has a specific activity for the toxin that is half that of the susceptible Betzes (table 1).

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